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HLA-DRB allels, IL-10 and Vitamin D level: Potential Impact on Multiple Sclerosis

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Abstract

Background Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease with unknown etiology. Variation in the HLA-DRB1 gene is the potent genetic risk factor for promoting MS. Other agents relate with an increased risk of developing MS include the cytokines levels such as interleukin-10 (IL-10) and the vitamin D (vit. D) deficiency.

Objectives To study a possible role of HLA-DRB1, vit. D deficiency as a risk factor for MS development and to estimate the level of IL-10 in the serum of MS patients and its role in disease initiation or progression.

Methods Sixty MS patients, of them 30 were newly diagnosed with an age range between 13 and 58 years were included in this study, in addition to thirty healthy volunteers their gender and age matched with patients group serve as a control group. Blood samples collected to assess serum levels of vit. D and IL-10 by Enzyme-Linked Immunosorbant Assay (ELISA) and for DNA extraction, which used in the HLA-DRB1 2 digit genotyping.

Results The HLA-DRB1 genotyping revealed that the HLA-DRB1*15 frequency was higher but statistically insignificant in the MS patients as compared with healthy control group. IL-10 level was significantly lower in MS patients on treatment than the control group. On the other hand, vit. D in the newly diagnosed MS patients was significantly different from the control group (higher in the control group), but there was no variance with MS patients on treatment, the level of vit. D in the studied groups was less than global value.

Conclusion In MS patients the frequency of HLA-DRB1*15 was higher than control group but the difference was not significant. In addition, the level of IL-10 and vit. D may have a role in the development of MS.

Keywords Multiple sclerosis, human leukocyte antigen, IL-10

List of Abbreviation: MS = multiple sclerosis, HLA = human leukocyte antigen, IL-10 = interleukin 10, vit. D = vitamin D.

Introduction

Multiple sclerosis (MS) is a complex autoimmune disease, not referable to a single genetic or environmental factor [(1)]. Several diseases with features common to MS are associated with certain human leukocyte antigen (HLA), especially autoimmune diseases [(2)]. HLA system provides a set of genetic loci their proteins play important role in immune response. Several studies indicated an association between the alleles of the HLA-DRB1 and MS. In northern European-descended populations, association with this gene was identified only within families that carried the HLA DRB1*1501 allele [(3)]. The family history of MS is a strong known risk factor, individual has an approximately 1-in-750 (0.1%) chance of developing MS. The first-degree relatives of the person with MS have approximately 2.5% to 5% risk factor to develop MS, while the identical twins of

patients with MS have a 25% chance of developing the disease [4].

Since MS is an autoimmune disease, there may be important role of cytokines such as interleukin-10 (IL-10) in MS development or course of the disease. High levels of Th1 cytokines are mostly obvious during experimental autoimmune encephalomyelitis and MS relapse, but there are high levels of Th2 cytokines during remission in MS patients [5].

Vitamin D (vit. D) can modulate the innate and adaptive immune responses where its deficiency is associated with increased autoimmunity as well as an increased susceptibility to infection. There is growing epidemiologic evidence linking vit. D deficiency and autoimmune diseases like MS, diabetes mellitus, and rheumatoid arthritis (6).

The objective of this study was to study a possible role of HLA-DRB1, vit. D deficiency as a risk factors for MS development and to estimate the level of IL-10 in the serum of MS patients and its role in disease initiation or progression.

Methods

A present case control study involved sixty MS patients with an age range from 13 to 58 years who were seeking treatment or those attending for the diagnosis in the MS Outpatients' Clinic at Baghdad Teaching Medical City in the period from December 2014 to March 2015. The diagnosis of each case was established according to MC Donald criteria done by a neurologist and confirmed by MRI and sometime by oligo-clonal band test in the CSF.

Patients were divided into two groups; group I on treatment and group II as newly diagnosed patients. This study was approved by the Institutional Review Board of the College of Medicine, Al-Nahrain University, and all samples were obtained with informed consent in accordance with the Teaching Hospital of Medical City declaration.

Five ml of blood were collected from each patient and control, divided into 3 ml for serum separation and 2 ml for DNA extraction then stored at -20 ^{o}C until used.

DNA extraction:

DNA Extraction Kit (Genaid) is optimized for genomic, mitochondrial and virus DNA purification from whole blood (fresh blood and frozen blood) and tissue.

Detection of vit. D in the serum (Eurouimmun – Germany)

This ELISA test kit is designed for the in vitro determination of 25-OH vit. D in human serum or plasma samples.

Detection of human IL-10 (Ray Bio – USA)

Human IL-10 ELISA kit is an in vitro enzyme-linked immune-sorbentassay for the quantitative measurement of human IL-10. This assay employed an antibody specific for human IL-10 coated on a 96- well plate. Standards and samples are pipetted into the wells and IL-10 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IL-10 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IL-10 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

HLA-DRB1 genotyping (Fujirebio – Belgium)

The HLA-DRB1 genotyping is done by INNO-LIPA HLA-DRB1 amplification plus kit and INNO-LIPA HLA-DRB1 plus kit (which is line probe assay) by single sequencing probe, PCR-SSO method.

Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-22

(Statistical Packages for Social Sciences- version 22). Statistical significance was considered whenever the P value was equal or less than 0.05. The significance of difference of different means (quantitative data) were tested using Students-t-test for difference between two independent means or Paired-t-test for difference of paired observations (or two dependent means), or ANOVA test for difference among more than two independent means. The significance of difference of different percentages (qualitative data) was tested using Pearson Chi-square test with application of Yate's correction or Fisher Exact test whenever applicable.

Result

The mean age of patients was 34.5 ± 10.6 years, and there was female predominance among patients, there were 35 (58.3%) of MS patients females, while 25 (41.7%) were males. Regarding distribution of patients according to family history the current data showed that (10.0%) of patients had positive family history of MS. No statistically significant association ($p > 0.05$) in age or gender existed between patients and control group.

The MS patients were distributed on three subgroups according to type of the disease, 55 (91.6%) of patients were with relapsing remitting type and 3 (5%) of patients were with secondary progressive type while 2 (3.3%) of patients with primary progressive type. The HLA genotyping was done for (48) patients and (30) controls, the result referred to considerable difference (but not significant $P > 0.05$) between the patients and control regarding DRB1 *15, table (1), 12 out of 48 patients were DRB1 *15 positive and 3 out of 30 controls were positive (P value 0.102). The DRB1 *11 was high in both groups (18 in MS group and 11 in control) but no significant difference between them. Also DRB1 *13 was high in the 2 groups without significant difference (12 in MS 11 in controls).

Table 1. HLA-DRB1 genotypes distribution among cases and controls

Allel	Multiple sclerosis		Control		OR	95% CI for OR	*P* value
	Positive	%	positive	%			
DRB1*01Allel	2	4.2	2	6.7	0.609	0.081-4.568	0.626
DRB1*03Allel	14	29.2	13	43.3	0.538	0.208-1.397	0.201
DRB1*04Allel	10	20.8	4	13.3	1.711	0.484-6.044	0.401
DRB1*06Allel	-	-	1	3.3	-	-	-
DRB1*07Allel	11	22.9	7	23.3	0.977	0.331-2.880	0.966
DRB1*08Allel	3	6.3	1	3.3	1.933	0.192-19.49	0.570
DRB1*09Allel	2	4.2	-	-	-	-	-
DRB1*10Allel	2	4.2	2	6.7	0.609	0.081-4.568	0.626
DRB1*11Allel	18	37.5	11	36.7	1.036	0.403-2.666	0.941
DRB1*13Allel	12	25.0	11	36.7	0.576	0.214-1.548	0.272
DRB1*14Allel	2	4.2	2	6.7	0.609	0.081-4.568	0.626
DRB1*15Allel	12	25.0	3	10.0	3.0	0.770-11.69	0.102
DRB1*16Allel	2	4.2	-	-	-	-	-
Blank	6	3					

OR = odd ratio, CI= confidence interval of OR

The present study showed significant decrease ($P < 0.05$) in the serum level of IL-10 among MS patients (1.49 pg/ml) as compared with healthy control group (1.71 pg/ml). Similarly, the vit. D

level was insignificantly less in patients group (13.02 ng/ml) as compared to 15.31 ng/ml of the control group (Table 2). Considering the IL-10 level, the current result revealed no significant differences between healthy controls and newly diagnosed MS patients, while it was significantly less (*P* = 0.009) in the treated MS patients versus healthy control group. Similarly, its level was significantly decreased (*P* = 0.028) in the treated versus newly diagnosed MS patients. Interestingly, comparing the three studied groups showed significant difference in mean of vitamin D level between control group and newly diagnosed patients (Table 3).

Likewise, no significant differences between healthy control and MS patients on treatment. In addition, the present study showed no significant correlation between IL-10 and vit. D, (Table 4).

Table 2. Interleukin-10 and vitamin D levels in patients with multiple sclerosis and control group

Parameter	Multiple sclerosis N = 60			Control group N = 30			*P* value
	Mean ± SD	SE	Range	Mean ± SD	SE	Range	
IL-10 (pg/ml)	1.49±0.40	0.054	0.60-3.10	1.71±0.56	0.114	1.10-3.40	0.049*
Vit. D (ng/ml)	13.02±5.89	0.794	4.50-35.50	15.31±6.01	1.203	4.60-31.00	0.114

IL-10 = interleukin-10, Vit. D = vitamin D, *Significant difference using student-t-test between two independent means at 0.05 level

Table 3. Interleukin-10 and Vit. D levels in the serum of MS sub groups and controls

Group	IL-10 (pg/ml)			Vit. D (ng/ml)		
	Mean ± SD	SE	Range	Mean ± SD	SE	Range
Newly MS	1.60±0.44	0.080	1.10-3.10	11.04±4.32	0.831	4.50-18.80
Treated MS	1.37±0.32	0.062	0.60-2.30	14.93±6.61	1.248	5.20-35.50
Control group	1.71±0.56	0.114	1.10-3.40	15.31±6.01	1.203	4.60-31.00
***P* value**		0.009*			0.005*	
***P* value**		0.028‡			0.013‡	
***P* value**		0.022†			0.014†	

IL-10 = interleukin-10, MS = multiple sclerosis, * = significant difference using student-t-test comparing treated MS patients with the control group, ‡ = significant difference using student-t-test comparing treated MS patients versus newly diagnosed MS patients, † = significant difference using ANOVA test among three independent means.

Table 4. Correlation between IL-10 and vitamin D in MS subgroups

Group	r	p
MS (n=60)	-0.110	0.434
Newly MS (n=30)	-0.051	0.801
Treated MS (n=30)	-0.011	0.957

* IL-10 (pg/ml), vit. D (ng/ml)

Discussion

Current result revealed higher frequency of DRB1 *15 allele in MS group as compared with control, the difference was statistically not significant but considerable which is agreed with Chao et al [7], Hossein et al [8] and disagree with Disanto et al [9]. As it is known the common HLA alleles are different between the populations depend on the ethnic group, migration and other factors. The allele

frequency of HLA-DRB1*15 is found to be around 33-36% among Norwegian MS patients, significantly higher than control [10]. In most European studies on MS populations there is an association to the HLA-DRB1*1501 allele [11]. It has been suggested that there is another HLA DRB1 allele associated with the increase susceptibility for MS, the most determined additional risk allele is HLA-DRB1*03 [12]. In Sardinia, the HLA-DRB1*03 is significantly associated with MS while HLA-DRB1*1501 has a low frequency [13]. It is estimated that the HLA class II association accounts for 20-60% of the genetic susceptibility in MS [14].

In addition, the current results revealed that the IL-10 concentration in treated MS patients was significantly less than healthy control, this result correspond with other studies Hasheminia et al [15] and Inoge et al [16]. The level of IL 10 in treated MS patient with interferon beta was less than of newly diagnosed MS patients this result agree with Inoge et al [16] and disagree with Oèzenci et al [17] who referred that the untreated patients had lower numbers of IL-10 secreting blood MNC(mono nuclear cell) compared with the control group. Dimisianos et al [18] referred that the IL-10, IL-4 and IL-6 not affected by IFNβ treatment, so, the significantly decrease of IL-10 in the treated patient may be not due to the treatment, but may be during the disease progression there will be depletion in the IL-10 which lead to relapses recurrence. Peelen et al [19] mentioned that IL-10 not affected with treatment in MS patients.

Interleukin 10 was first identified as a molecule that limits inflammation and supports humeral immune responses. IL-10 deficient animals develop lethal inflammation of the intestine, which can be relieved by ectopic administration of IL-10. Deficiency or aberrant expression of IL-10 can enhance inflammatory response to microbial challenge but also lead to development of a number of autoimmune diseases [20]. IL-10 has been also implicated in a number of other inflammatory animal models, including experimental autoimmune encephalomyelitis [21]. Thus, impaired IL-10 expression or signaling can enhance clearance of pathogens during an acute infection but also exaggerate inflammatory response resulting in exacerbated immunopathology and tissue damage [22].

Other studies have shown that increased IL-10 levels in spinal cord correlate with EAE remission, and exogenous administration of IL-10 effectively ameliorated EAE when targeted directly to the CNS. Similarly, lower production of IL-10 in humans appears to be a risk factor for MS, as accumulative data have shown that MS patients had lower IL-10-secreting T cell frequency than controls. Overall, experimental findings indicate that IL-10 has an important disease suppressor function in both EAE and MS [23-24].

In the present study, the level of vit. D in serum of patients and healthy controls was less than 30 ng/ml except one patient and one control. There was no significant deference's between healthy group and patients this result consistent with Al-Mahdawi et al [25]. Grau-López et al [26] showed no differences in the level of vit. D in healthy control and patients in summer and no relationships were found between plasma 25(OH) D concentrations and clinical or radiological variables. Present result disagree with Van der et al [27] and Soilu-Hänninen et al [28] who mention that the serum levels of vit D were significantly lower in patients than in controls .

Its worthy to mention that the level of vit. D in serum of newly diagnosed patient was significantly less than the healthy control. The explain of this result one of two probability: the first one is that the newly diagnosed patients usually have the first relapse maximally before 4-6 weeks and a lot of studies refer to that the concentration of the vit. D decreased during and after short period of the relapse according to Al-Mahdawi et al [25]. Also researchers working in Tasmania reported an inverse relationship between the relapses rate and estimated serum 25(OH) D [29].

Second explanation that the low level of vit. D is one of the environmental risk factors, which involved in the initiation of the disease, and the level of the vit. D in MS patients on treatment more than newly diagnosed patients may due to their listening to their doctor's advising to improve their nutrition or due to consumption of some of the nutritional complementary, which contain vit. D.

Many reports of vitamin D deficiency predicting development of autoimmune disease in the future have been published for MS, and other autoimmune disease like diabetes mellitus and rheumatoid arthritis [30].

Also in current study, there was a significant relationship between low levels of vit. D and the gender, the result revealed that the women were significantly lower than men in the serum level of vit. D; this agree with Issa, 2007 [31] who found that the vit. D level is significantly lower in women's serum also found variation in vit. D level among seasons. This work is disagreeing with Johnson et al [32]. This difference in vit. D levels between men and women in Iraqi population may due to social religious reasons and behaviors which is impose on women to cover all the body and as a result they will exposed to the sun light less than men, or due to repeated pregnancy which is led to vit. D depletion in the body. Also there is another explanation; that the difference is due to the normal physiology of males and females, the hormonal changes during the normal Menstruation may play a role.

In conclusion, the result of current study revealed that the HLA-DRB1*15 in MS patients was common but its cant be considered as a susceptible allele. As well as the IL-10 deficiency may play an important role in MS disease progression and its continuous deficiency may lead to increase disease activity. In addition, vit. D deficiency may play a role as cofactor for disease initiation or its role may restrict during relapses.

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Author Contribution

Dr. Abbas supervisor the study; Dr. Khaliel did the laboratory work and write the article; and Dr. Shaheed examined the patients and consultant.

Conflict of Interest

No conflict of interest

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